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Proliferative Diseases of Hemocytes, Endothelial Cells, and Connective Tissue Cells in Mollusks[1]

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Introduction

Several non-invasive tumorous growths have been described from bivalve mollusks. These have been reviewed by PAULEY [6]. Only recently have invasive proliferative disorders been described [1, 3, 4, 8, 9, 10]. PAULEY *et al.* [8] reported an invasive ganglioneuroma in a specimen of *Crassostrea gigas* and WOLF [10] described an epizootic of grossly detectable mantle epitheliomas in *C. commercialis* in Australia. The disorders reported by FARLEY in *C. virginica, C. gigas,* and *Mytilus edulis* [3, 4]; by COUCH in *C. virginica* [1]; by JONES and SPARKS [5]; and SPARKS *et al.* [9] in *Ostrea lurida* are the only molluscan neoplasms which have leukemia-like features. Although PAULEY and SAYCE [7] described a non-invasive fibrous tumor in *C. gigas* which appeared to consist of reticular type cells, no mitoses were observed.

In our pursuit of disease studies more than 100,000 specimens of west coast mollusks have been examined histologically since 1958 in the oyster pathology program at the College of Fisheries and more than 40,000 specimens have been examined since 1961 at the BCF Laboratory. Most specimens were oysters *(Crassostrea virginica, C. gigas, Ostrea lurida* and *O. edulis)* but recently two species of mussels *(Mytilus edulis* and *M. californianus)* have been studied.

About 40 cases of leukemia-like 'neoplasms' have been found since 1961. The occurrence of these disorders is rare in the genus *Crassostrea* [3]. However, a prevalence of 12% has been found in both *M. edulis* [4] and *O. lurida* from Yaquina Bay, Oregon [9].

[1] A joint contribution of the Bureau of Commercial Fisheries Biological Laboratory, Oxford, Maryland, and the College of Fisheries.

This report reviews the occurrence of leukemia-like diseases in mollusks, proposes a preliminary classification of cytologic types, describes newly found neoplasms in *C. virginica*, *C. gigas*, and *O. lurida*, and reports additional information concerning the histological origin of these suspected neoplastic cells.

Pathology and Occurrence

Gross features. Although a number of gross tumors have been described [6], only 2 have been diagnosed microscopically as hematopoietic disorders. Figure 1 is an illustration of a grossly apparent tumor found in *O. lurida*. Poor condition, pale-appearing digestive glands, and mantle recession have been correlated with an epizootic, fatal disease in oysters caused by infection by a protozoan parasite, *Minchinia nelsoni* [2]. These gross signs are indicative of starvation with resulting emaciation and mortality. One or more of these characteristics are usually present in advanced cases of neoplastic disease in oysters and mussels [3, 4].

The apparent rarity of grossly detectable invasive tumors in pelecypods could be due to several factors. The pelecypod circulatory system is semi-closed, with the hemolymph entering the arterial system from the ventricle and emptying into a number of large sinuses in the connective tissue where hemocytic migration occurs. Hemolymph collects in large veins in the venous system and is eventually returned to the auricles of the heart. The connective tissue in these animals consists of large vesicular cells surrounded by reticulum fibers which lend little actual support to the tissues. The nature of the circulatory system and the weak structural development of connective tissue is conceivably conducive to rapid dissemination of primary neoplasms before they become grossly apparent.

Cytology. Hemocytes in pelecypods occur as two major types: large (15 μ) granular phagocytes with nuclei averaging about 3 μ in diameter and smaller (8 μ) hyaline forms with nuclei which average about 4–5 μ in diameter. The cells involved in proliferative disorders can be classified cytologically into two types. The most easily recognized type (which has been found in *C. virginica*, *O. lurida*, and *M. edulis)* is characterized by enlarged cells and nuclei 2 to 4 times the size of normal hemocytic cells and nuclei (fig. 2, 5, 6, 8). Abnormal features often associated with these enlarged cells are: irregularities of nuclear shapes and generally irregular (or variable) contours; binucleate cells (multinucleate cells are found rarely) and increased density of chromatin; diffuse patterns of chromatin and nuclear membranes; multi-

ple nucleoli; and pyknosis and nuclear lysis in advanced lesions. Mitotic abnormalities are apparent as tripolar figures, chromosomes displaced from the mitotic spindle, and polyploidy. There is some evidence indicating that these neoplastic cells in *O. lurida* (fig. 5, 6) are de-differentiated vesicular connective tissue cells. Local lesions consisting of enlarged cells of *C. virginica*[2] 2, 3 [1, 3, 4] found in the connective tissue localized mantle tumor may also be neoplastic connective tissue cells. The lesion described by COUCH [1] and the most recently found disorder in *C. virginica*[3] (fig. 4) seem to be associated with the endothelial lining of hemolymph vessels and consist of enlarged, partially differentiated pleomorphic, and spindle-shaped cells. The most recent tumor had areas in which newly-formed hemolymph vessels were apparent and reticulin borders around many of the neoplastic cells. This tumor is being tentatively interpreted as an hemangioendothelioma.

The second cell type involved in leukemoid disorders is closely similar in size and morphology to hyaline hemocytes (fig. 3, 4). This disorder is thought to be neoplastic because it produces a highly invasive infiltration of most tissues, especially the vesicular connective tissue which is destroyed by the invasion. Mitoses are common in these cells and necrosis occurs in massive lesions in late stages. Heavy concentrations of RNA occur in the cytoplasm of these cells. Nuclei tend to be uniform in size and rounded although atypical nuclear configurations do occur. This hyaline hemocytic type of disorder has been found mostly in *C. virginica* (fig. 3); however, several cases have been found in *C. gigas* and one case in *O. lurida* (fig. 7).

Histopathology. Histologically these disorders are characterized by diffuse collections of abnormal, mitotically active cells, which invade and re-

[2] NEWMAN, M.W.: An invasive 'neoplastic' disorder in *C. virginica* from New Haven Harbor, Connecticut, Personal communication (1969).

[3] An undescribed 'neoplastic' lesion recently found in a specimen of *C. virginica* from Chesapeake Bay.

Plate 1

Fig. 1. Gross tumor (arrow) in mantle of *O. lurida.*

Fig. 2. Mitotic and interphase enlarged 'vesicular type' cells in *C. virginica* (see footnote[2]) disorder. 1000×.

Fig. 3. Mitotic and interphase 'hemocytic type neoplastic' cells in *C. virginica.* 1000×.

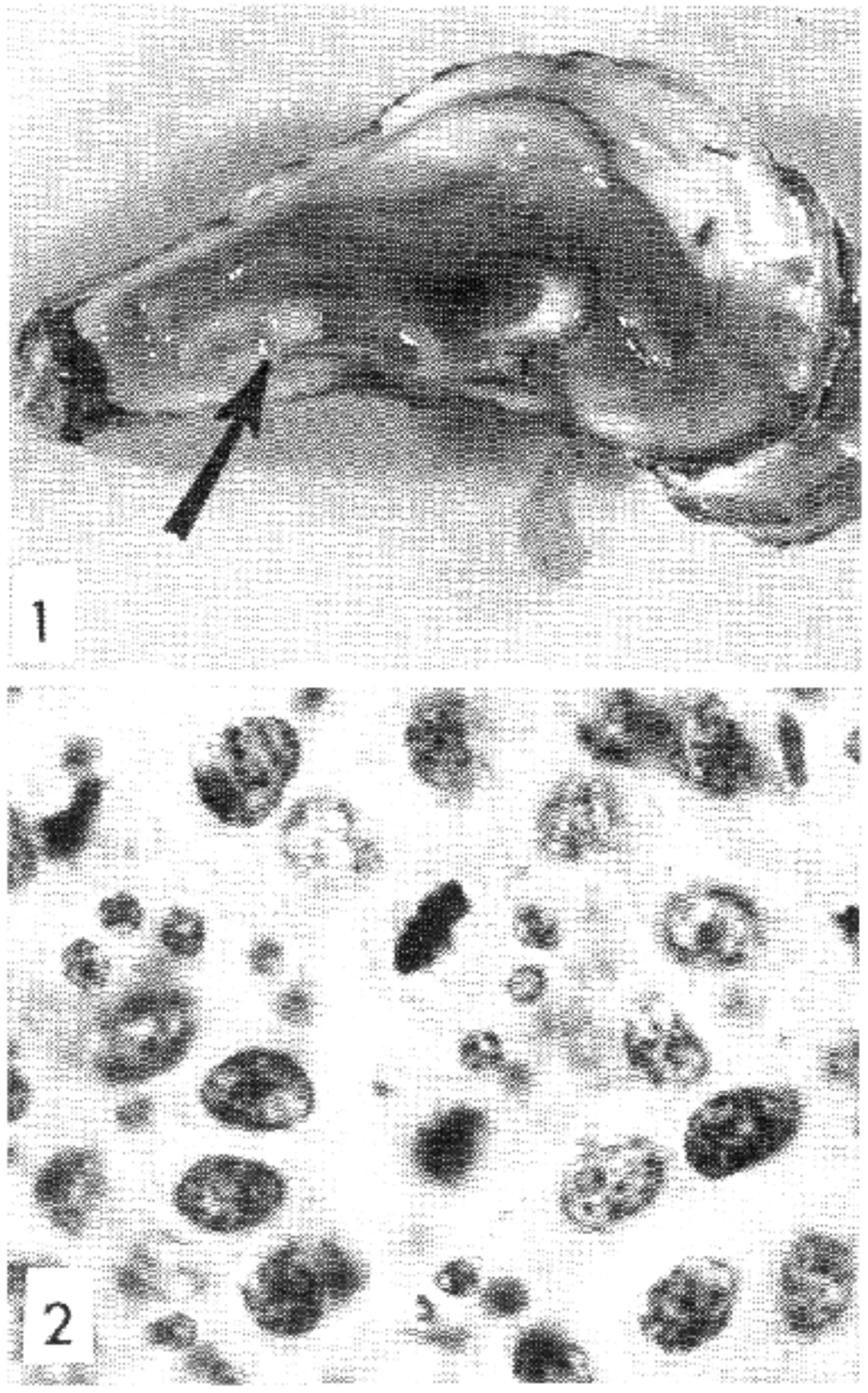
Fig. 4. 'Endothelial type neoplastic' cells in *C. virginica.* 1000×.

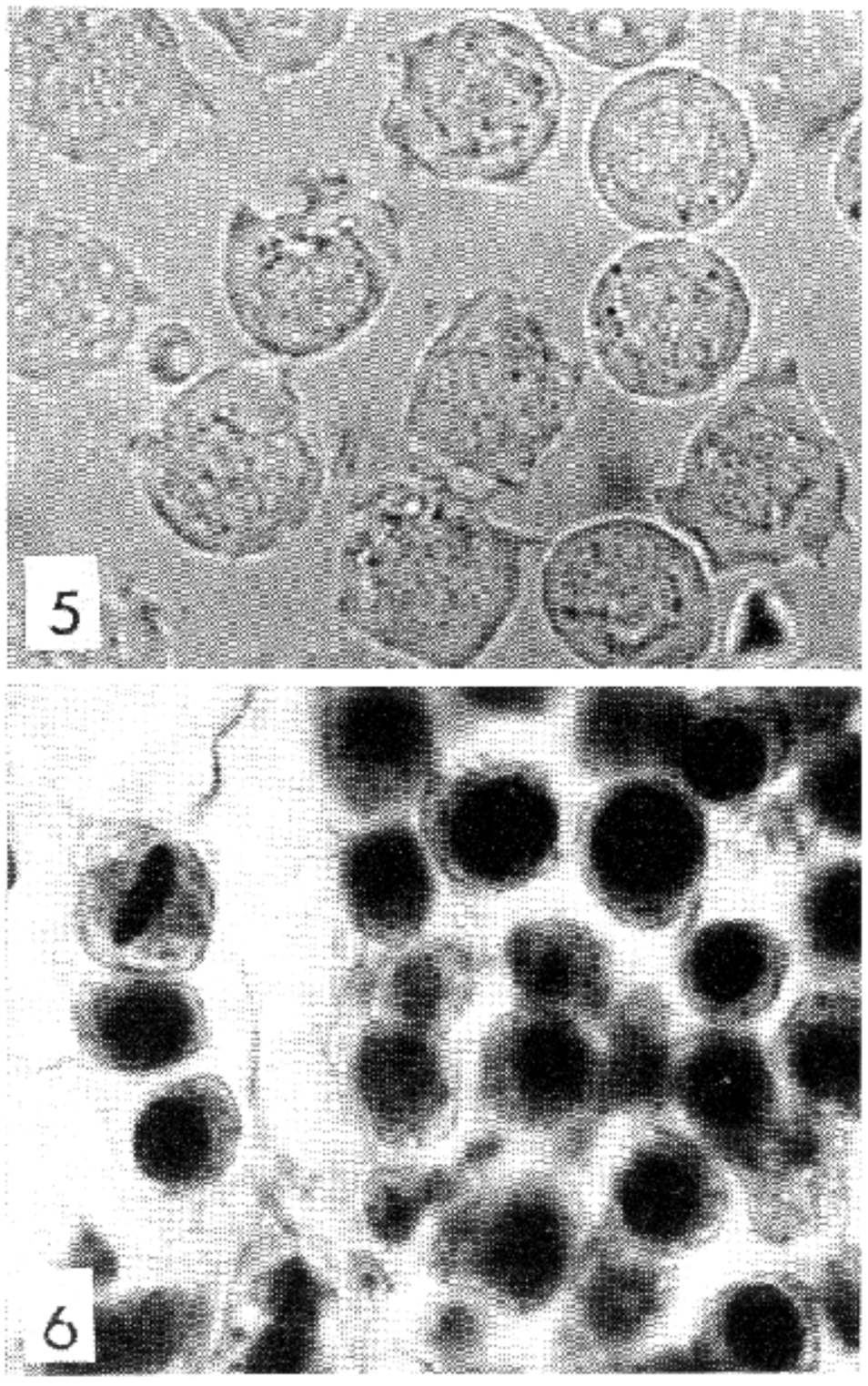
Fig. 5. In vivo 'vesicular type neoplastic' cells in *O. lurida.* 1000×.

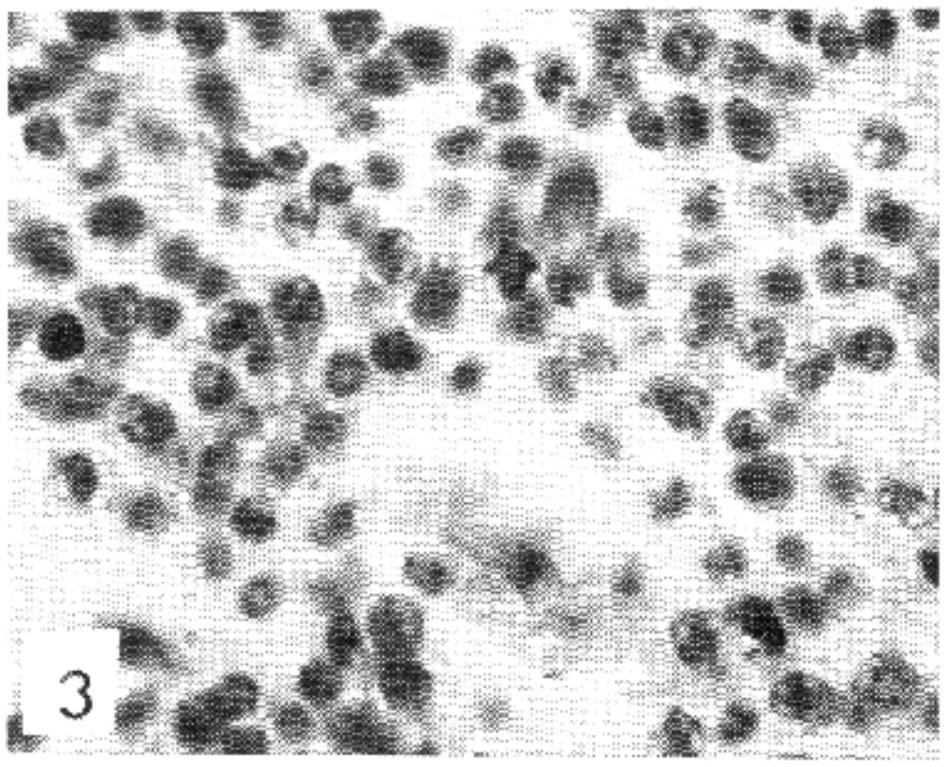
Fig. 6. Mitotic and interphase 'vesicular type neoplastic cells,' in section of *O. lurida.* 1000×.

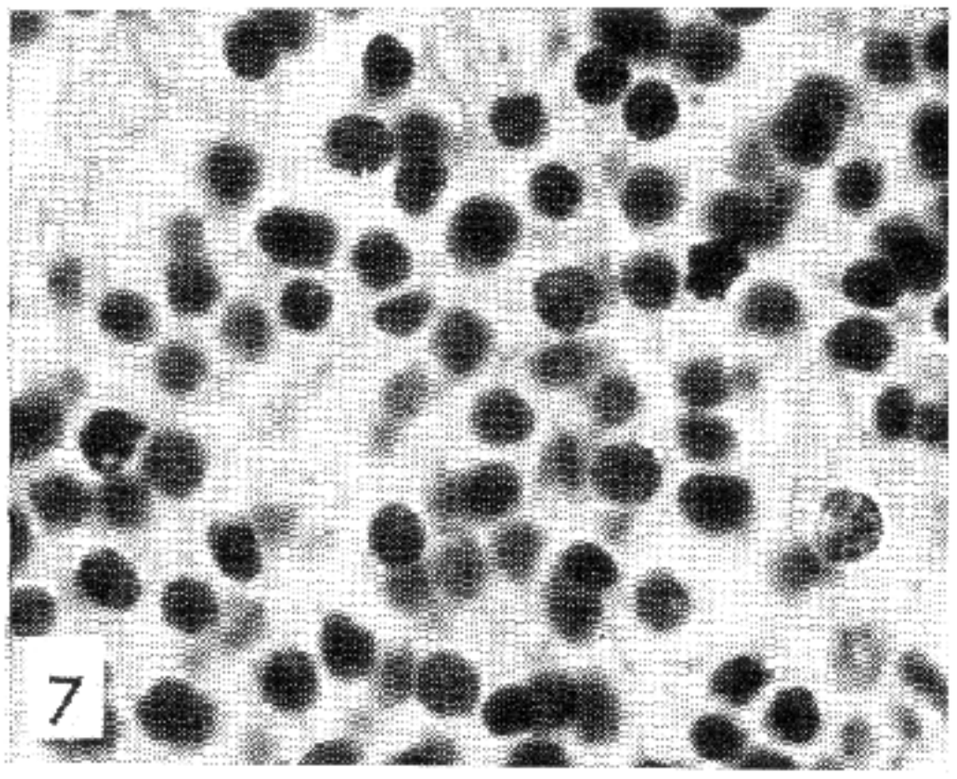
Fig. 7. 'Hemocytic type neoplastic' cells in *O. lurida.* 1000×.

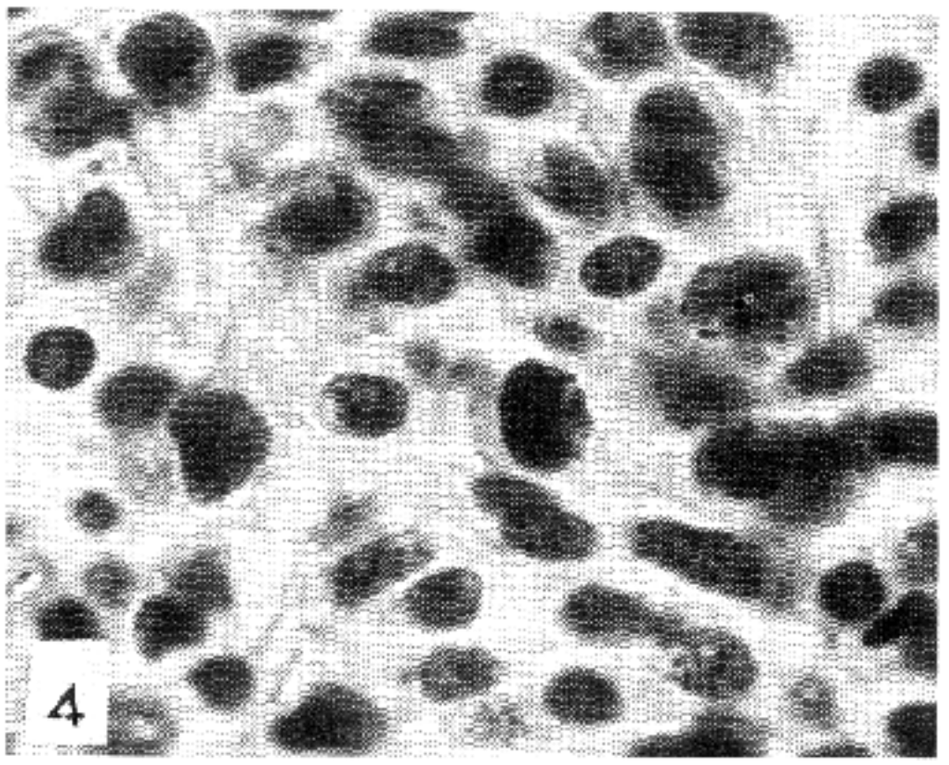
Fig. 8. 'Mitotic and interphase vesicular type neoplastic' cell in *M. edulis.* 1000×.











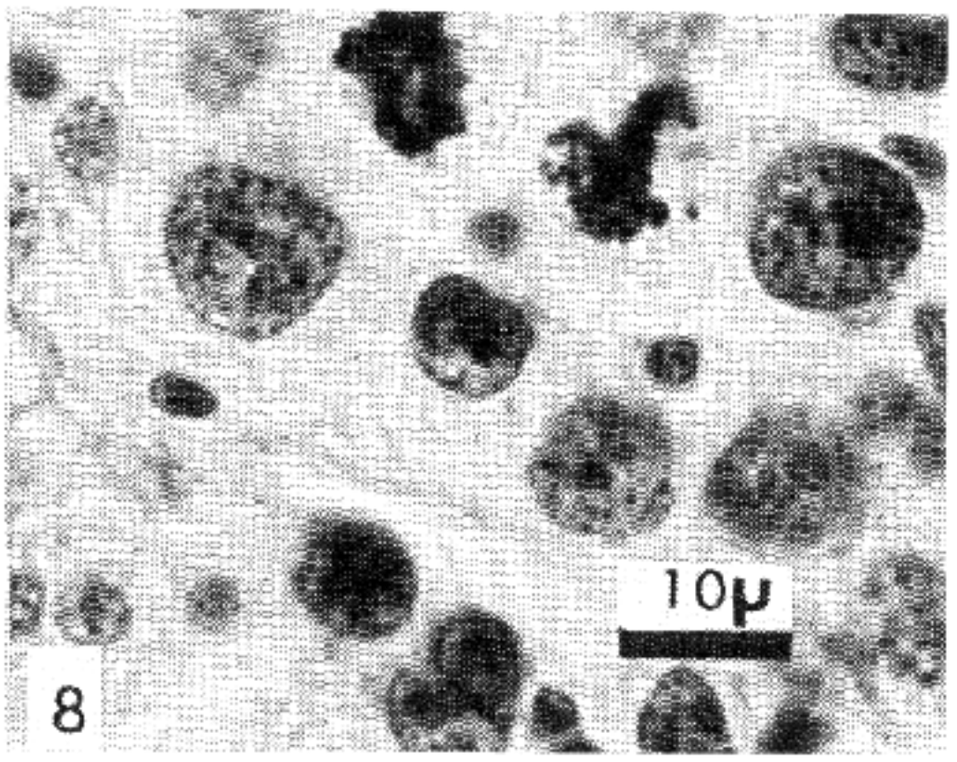


Plate I

place the vesicular connective tissue (fig. 9–14). Collections are sometimes evident first in the hemolymph vessels and sinuses, and can be found commonly in the muscle and byssal tissues of mussels. Invasion of epithelia is uncommon, but has been observed in *C. virginica* and *O. lurida*.

The disease in some mollusks *(M. edulis* and *C. virginica)* appears to originate as a local lesion in either the vesicular connnective tissue or the endothelial lining of hemolymph vessels. The earliest cases are characterized by one or more relatively large microscopic lesions with diffuse dissemina-tions of individual or small collections of 'neoplastic' cells in the hemolymph sinuses, lumina and endothelial linings of vessels, or in the vesicular connective tissue, as widely disseminated cells distributed throughout the hemolymph spaces. An intermediate phase is characterized by the development of extensive localized lesions in the vesicular tissue and gonad. Advanced cases show virtual universal dense infiltration of the connective tissue and other organs (fig. 9–14) and the complete arrest of gametogenesis. Gross pathology is usually evident in this stage. Terminal cases exhibit widespread degenerative changes in neoplastic and eventually normal tissues. The comparative histopathology of these disorders can be seen in figures 9–14.

Occurrence and seasonal aspects. Histologic examination of over 30,000 specimens of *C. virginica*, from the east coast waters of the United States, suggest that these 'neoplastic' disorders are quite rare (0.02%) in this species [3]. Several possible 'neoplasms' have been found in *C. gigas*, but further characterization and additional sampling is necessary to determine prevalence. In both *M. edulis* [4] and *O. lurida* from Yaquina Bay, Oregon, a peak prevalence of 12% occurred in December. Examination of three 25-specimen samples collected in September, December, and April indicated a seasonal pattern of occurrence with early cases appearing in *O. lurida* in the spring and in *M. edulis* in September. Advanced cases were most common in the early winter (December) and both species showed apparent indications of mortality during this period.

Plate 2

Fig. 9. 'Hemocytic neoplasm' in vesicular connective tissue of *C. virginica*. 250×.

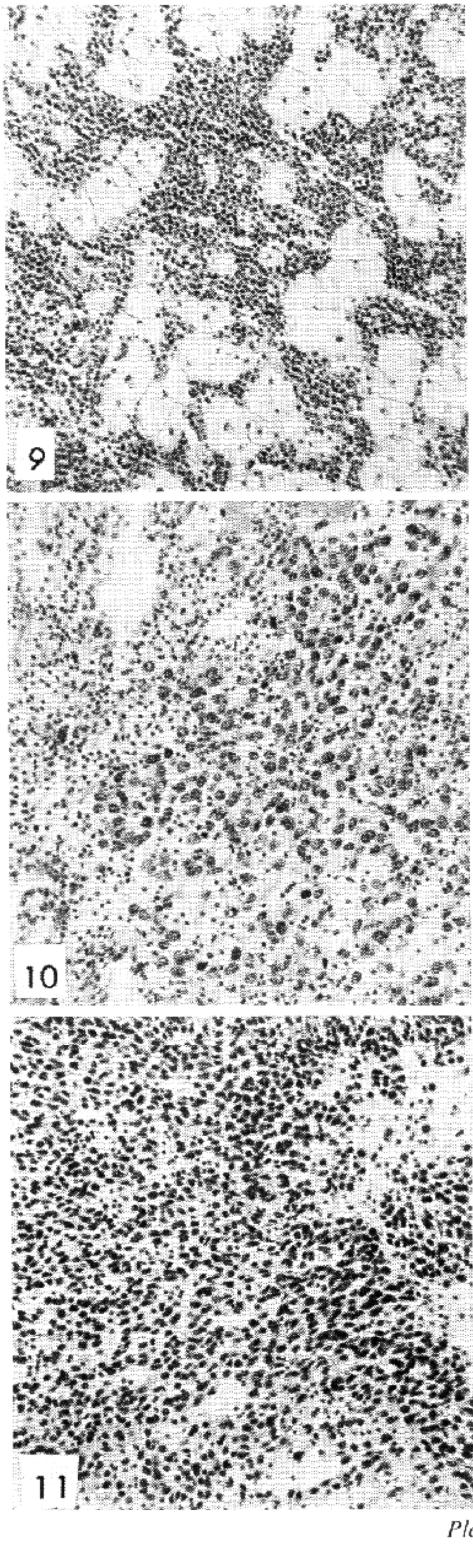
Fig. 10. 'Vesicular cell neoplasm' in gill of *C. virginica* (see footnote [2]). 250×.

Fig. 11. 'Endothelial neoplasm' in *C. virginica*. 250×.

Fig. 12. 'Hemocytic neoplasm' in vesicular connective tissue of *O. lurida*. 250×.

Fig. 13. 'Vesicular cell neoplasm' in *O. lurida*. 250×.

Fig. 14. 'Vesicular cell neoplasm' in *M. edulis*. 250×.



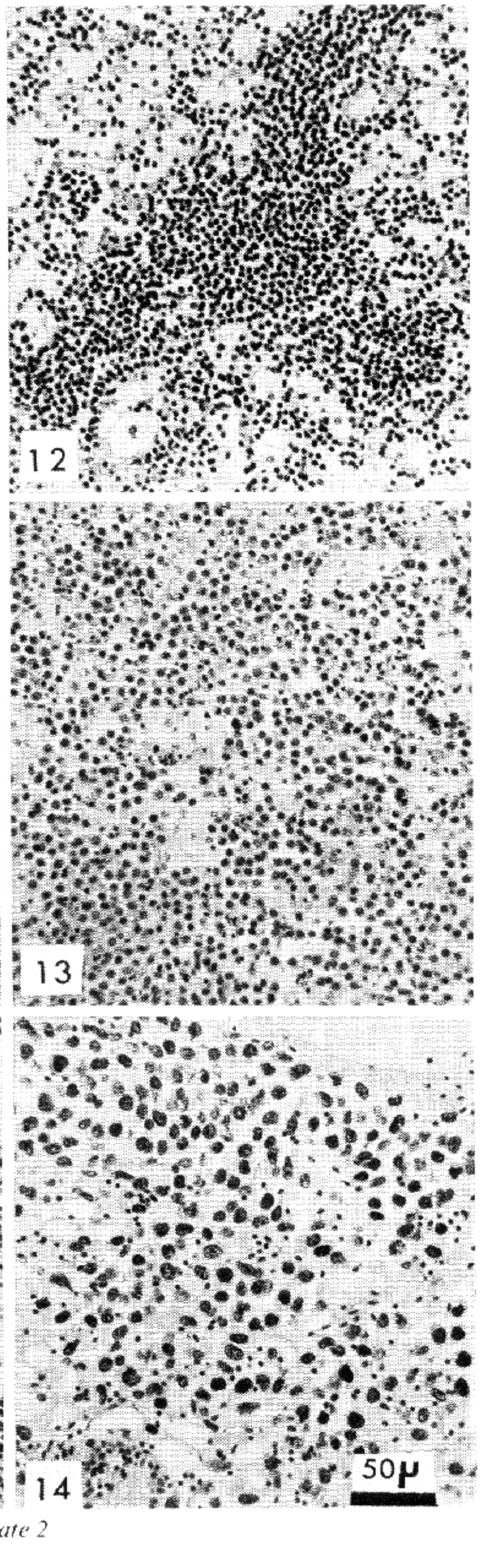


Plate 2

Discussion

These recently described disorders of pelecypod mollusks exhibit many of the general criteria recognized for neoplasia in vertebrates—proliferation of a single line of cell, unrestricted infiltration, nuclear and mitotic abnormalities, and morbid, gross and histologic changes indicative of fatal outcome. Transplantation-transmission experiments and ultra-structure studies in progress have yet to yield definitive results. Recent findings concerning the origin and development of the three cell types involved have provided additional evidence concerning their autochthonous nature. However, without experimental evidence, a definitive interpretation of neoplasia cannot be made. The pathology, cytology and developmental sequences are most suggestive of neoplasia, but parasitic involvement cannot yet be eliminated.

The seasonal nature of the onset of these abnormalities is characteristic of virtually all known oyster epizootics of parasitic causation. This led the junior author and his associates to suspect a parasitic involvement in the *O. lurida* abnormalities for several years. However, no protistan of known pathogenicity to oysters has been found associated with the diseased oysters. It is, of course, possible that the abnormal cells described are themselves a parasite of the mollusk rather than neoplastic cells. Their morphology and mitotic activity are, however, unlike any known parasitic forms, but are strongly suggestive of molluscan cells.

The etiology of these disorders remains obscure at present and speculation on the causation would be unwarranted. Possible initiating agents include, but are not confined to, viral infection, industrial or domestic pollution, low salinity, genetic factors, or unrecognized parasites. It must be pointed out, however, that conditions of low salinity and pollution exceeding those found in the locations from which these abnormal mollusks were collected either do not develop similar pathoses or the apparent neoplasms have not been recognized.

Summary

Probable neoplasia involving the hematopoietic or reticular tissues have been found in oysters *(C. virginica, C. gigas,* and *O. lurida)* and mussels *(M. edulis)*. The disorders are rare (0.02% in crassostreids from both US coasts, but epizootic (12%) in ostreids and mytilids from an Oregon bay. The diseases are characterized by local diffuse lesions consisting of undifferentiated, proliferative, apparently invasive, hemocytoid cells which often show abnormal cytologic features. Early, intermediate, advanced, and terminal phases are recognized. Gross pathology is apparent in specimens with advanced stages of disease and

a fatal outcome is indicated. Two or more cytologic types are apparent and connective tissue, endothelium, and hematopoietic origins are suspected. The concept that these disorders represent leukemic neoplasia is favored. However, the possibility of parasitic involvement cannot yet be completely eliminated.

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